Amendments to the Specification:

After the title of the invention on page 1, please add the following section:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. Application No. 09/701,413, filed February 20, 2001, which claims priority to the National Phase Entry of PCT/US99/28769, filed December 7, 1999, which claims priority to U.S. Provisional Application No. 60/111,195, filed December 7, 1998, all of which are hereby incorporated herein in their entirety by reference.

Please replace the paragraph on page 3, lines 2-14, with the following paragraph:

The present invention relates to a novel reagent and method for isolating stem cells, including human stem cells. The reagent is a fluorescent substrate for ALDH. The method comprises staining a cell population that includes primitive stem cells with the substrate in the presence of an inhibitor of MDR activity. ALDH present in the cells converts the substrate to a product that is trapped within the cells. Since primitive stem cells have higher levels of ALDH activity than other cell types, these cells stain brighter than other cell types. The presence of the MDR inhibitor reduces the efflux of the converted substrate from the stem cells.

Please replace the paragraph on page 3, line 25, through page 4, line 5, with the following paragraph:

Figures 2A-2D. Converted BAAA is effluxed by an MDR pump from hematopoietic cells, particularly primitive CD34+ cells, as evidenced by the difference between the CD34+ cells that are $BODIPY^{bright}$ (Fig. 2B) in the presence and absence of the MDR inhibitor, verapamil (Figs. 2A and 2B are at t=0, minus and plus verapamil, respectively; Figs. 2C and 2D are at t=30', minus and plus verapamil, respectively.)

Please replace the paragraph on page 4, line 20, through page 5, line 4, with the following paragraph:

Figure 6. Preparation of ~~BAAA~~ BODIPY-aminoacetaldehyde diethylacetal. Using an amber vial, a solution of aminoacetaldehyde diethyl acetal (0.019 mmol, Aldrich Chemical Co.) in dry tetrahydrofuran (THF, 0.5 mL) was added dropwise to a solution of BODIPY FL, SE (0.013 mmol, Molecular Probes) in dry THF (0.5 mL). Upon complete addition, the vial was capped and the reaction mixture was stirred for 30 min. The THF was evaporated and the residue was dissolved in minimal methylene chloride and then chromatographed on silica gel using ethyl acetate - hexane (1:1) as eluent. The product, BODIPY-aminoacetaldehyde diethylacetal ~~(BAAA)~~ was recovered in quantitative yield and identified by proton NMR.

Please replace the paragraph on page 5, lines 6-17, with the following paragraph:

The present invention relates to a method of isolating stem cells and to a reagent suitable for use in such a method. The method comprises contacting a population of cells comprising stem cells with a detectable substrate for aldehyde dehydrogenase (ALDH), which substrate is converted to a detectable product by ALDH, that product being retained in the cells. In a preferred embodiment, the substrate is BODIPY-aminoacetaldehyde (BAAA) and efflux of converted BAAA from the cells, particularly the stem cells present in the population, is inhibited by the concurrent use of a MDR-inhibitor.

Please replace the paragraph on page 6, line 21, through page 7, line 2, with the following paragraph:

In order to inhibit efflux of the converted substrate of the invention from the cells, concurrent use of an inhibitor of MDR is preferred. Any of a variety of MDR inhibitors can be used, including verapamil. The inhibitor can be added to the cells simultaneously with the substrate or prior to the addition of the substrate. The optimum amount of MDR-inhibitor to be

used can be readily determined (e.g., by monitoring loss of staining). In the case of verapamil, concentrations can vary, for example, a concentration of about 50μM can be used.

Please replace the paragraph on page 8, lines 3-23, with the following paragraph:

Gene therapy approaches involving the present cells involve, in one embodiment, isolation of autologous stem cells, exposure of the isolated cells to a gene delivery vector and re-infusion of the modified cells into the patient (Smith, J. Hematother. 1:155 (1992)). This approach can involve *ex vivo* culture or the use of vectors capable of transferring genes into non-dividing cells, thereby rendering ex vivo culture unnecessary. Gene therapy can be useful in treating, for example, congenital diseases, such as sickle cell anemia, in which case the mutant ~~β-globin~~ β-globin gene is replaced or supplemented with either the wild type globin gene or an anti-sickling globin gene. In the treatment of cancer, drug resistance genes can be introduced into the stem cells to confer resistance to cytotoxic drugs. This can reduce the incidence and severity of myelosupporession. For the treatment of infectious diseases, including HIV, anti-viral genes can be introduced into the stem cells so that they are rendered resistant to the virus (Gilboa and Smith, Trends in Genetics 10:139 (1994)).

Please replace the paragraph on page 10, lines 22-29, with the following paragraph:

The invention also relates to kits that can be used to prepare the cells of the invention. The kits can comprise reagents (e.g., ALDH substrate) that can be used to effect isolation of the stem cells. In a preferred embodiment, the kit includes ~~BAAA~~ BODIPY aminoacetaldehyde diethyl acetal disposed within a container means. The kit can also include, disposed within a container means, an MDR inhibitor, such as verapamil.

Please replace the heading and paragraph on page 11, lines 6-23, with the following heading and paragraph:

Preparation of BODIPY aminoacetaldehyde diethyl acetal

The aldehyde dehydrogenase substrate is prepared as ~~BODIPY aminoacetal~~ BODIPY aminoacetaldehyde diethyl acetal and lyophilized in 0.5 micromole aliquots. These preparations are stable indefinitely when stored at -20°C. The acetal is then solubilized in DMSO to a final concentration of 5 mM. This solution has been found to be stable at 4°C for up to 1 week. To convert the acetal to an acetaldehyde, aliquots of this solution are brought to a final concentration of 1 N HCl. Under these conditions the acetal has a half life of 15 minutes. After 2 hours in 1 N HCl, the vast majority of the ~~BODIPY aminoacetal~~ BODIPY aminoacetaldehyde diethyl acetal has converted to BODIPY aminoacetaldehyde (BAAA), and is then diluted to 200-250 mM in Dulbecco's phosphate buffered saline (PBS). This stock is added directly to cells prepared in Iscove's Modified Dulbecco's Medium (IMDM) with 2% FCS at concentrations ranging from 1 to 5 μM.)(See also Fig. 6.).

Please replace the paragraph on page 14, line 17, through page 15, line 20, with the following paragraph:

Hematopoietic progenitor colony assays were performed by plating 100-200 cells in MethoCult H4431 containing agar leukocyte conditioned media and recombinant human erythropoietin (StemCell Technologies, Inc.). The cells were incubated in a humidified chamber at 37°C with 5% CO_2. Hematopoietic colonies (>100 cells) were then scored at 14 to 18 days after initiating the cultures. Long term cultures were maintained on stromal layers of murine MS-5 cells (provided by Dr. Tadashi Sudo of the Kirin Pharmaceutical Research Laboratory, Gunma, Japan) (Issaad, Blood 81:2916 (1993)). MS-5 stromal cells were seeded into 24-well plates (Corning Costar Corp., Cambridge, MA) at 5 X 10^4 cells/well in DMEM supplemented with 10% FCS and cultured at 37°C. When the monolayers approached 80% confluence they were γ-irradiated from a cesium source (40 Gy). After irradiation, fresh media was provided to the cultures. For the MS-5 cells, the culture media was replaced entirely with MEMα supplemented with 10% FCS,10% equine serum, β-mercaptoethanol, pyruvate. Long term cultures were initiated with 400-2000 hematopoietic progenitor cells/well and were maintained at

33°C with 5% CO_2. At weekly intervals half the media from each well was removed so that the media could be replenished. Adherent and non-adherent cells were harvested after 5 or 8 weeks and plated into HPC assays as described above. As shown in the Example that follows, sorting the brightest 1% of cells yields a nearly 40-fold enrichment for cells that initiate long term cultures ~~(how can higher levels of enrichment be achieved or would cell cloning be used at this point??)~~. The cell preparations that were recovered were up to 65% $CD34^+$ cells, most of which were CD34+ cells, most of which were $CD38^{-/dim}$ $CD71^{-dim}$.

Please replace the heading and paragraph on page 15, line 22, through page 16, line 6, with the following heading and paragraph:

Synthesis of ~~BODIPY acetal~~ BODIPY aminoacetaldehyde diethyl acetal.

Due to the inherent instability of aldehydes in aqueous solution, the reagent is prepared and stored as an acetal. Immediately prior to its use, the acetal is converted to an aldehyde in 1 N HCL. the aldehyde is freely soluble in PBS and can be added directly to cells prepared in IMDM with 2% fetal calf serum at 10^6 cells per ml. As an aminoacetaldehyde, the reagent is membrane permeable; however, in the presence of the aldehyde dehydrogenase (ALDH), the aldehyde moiety is converted to a carboxylic acid that is retained in the cell. Intracellular fluorescence can be used to select cells.

Please replace the heading and paragraph on page 16, lines 7-24, with the following heading and paragraph:

Converted BAAA is a Specific Substrate for ALDH.

To assay whether BAAA would permit the specific selection of $ALDH^+$ cells, studies initially determined an optimal response dose for the BAAA reagent in a murine cell line previously selected for cylophoshamide-resistance, L1210/cpa, that is known to be $ALDH^+$ (Fig. 1). The parental cell line, L1210 (Figs. 1A and 1B) is cylophosphamide-sensitive and $ALDH^-$. This cell line exhibited essentially no response to BAAA. In addition, a potent inhibitor of ALDH, diethylbenzaldehyde (DEAB), was used to demonstrate the specificity of the

BAAA signal. A 10-fold molar excess of DEAB totally blocked the fluorescent response (Fig. 1C). Therefore, BAAA was able to detect $ALDH^+$ cells. In these studies, the BAAA could be used at a final concentration as low as 5μM. This molar concentration is 10-fold lower than that used with the dansylated reagent.

Please replace the paragraph on page 10, lines 22-29, with the following paragraph:

In addition to expressing ALDH, PHSC should also express high levels of the P-glycoprotein or multiple drug resistance (MDR) efflux pump. Since this reagent had never been previously characterized, the susceptibility of converted BAAA to MDR efflux was assayed. Although BODIPY aminoacetaldehyde passes through the cell membrane without active transport, the product of the ALDH conversion (BODIPY aminoacetate) might well be a substrate for the MDR pump. To investigate this possibility, UCB cells were stained with BAAA in the presence of 50μM verapamil, a competitive inhibitor of the MDR efflux pump. The verapamil-treated cells exhibited a consistently-higher fluorescence when compared with BAAA-stained cells that had not been simultaneously treated with verapamil (Fig. 2). A substantial population of $ALDH^{dim}$ cells were effected by the verapamil treatment. Most importantly, the percentage of $ALDH^{br}$ cells increased by 1.8 fold in the presence of verapamil. In verapamil-treated cells, the $ALDH^{br}$ subpopulation was equivalent to 0.8∀% of the SSC^{lo} cells. In contrast, in cell preparations that received no verapamil, the same fluorescence intensity represented only 0.46±% of the SSC^{lo} cells. This indicated that the $ALDH^{br}$ SSC^{lo} UCB cells retain the converted BAAA more effectively if the efflux activity of the MDR pump is inhibited.